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Evaluation of phytochemical composition, toxicity in *Drosophila melanogaster* and effects on antibiotics modulation of *Plathymenia reticulata* Benth extract

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ABSTRACT

Bacterial resistance is interfering with the action of antibiotics for clinical use in treating pathologies. The search for new substances capable of combating this resistance is necessary. An alternative to the search for these substances is in the extract of medicinal plants. *Plathymenia reticulata*, plant of the Fabaceae family, is a common tree species from the Brazilian cerrado, and is commonly used in areas of environmental degradation. This species is rich in phenolic compounds, such as flavonoids and tannins, compounds that are associated with various biological effects. A hydroethanolic extract from the bark of *Plathymenia reticulata* (HEPrB) was produced and then tests were carried out to verify the direct antibacterial activity, the modulatory effect of antibiotics for clinical use and their toxicity in *Drosophila melanogaster* flies. Through the analysis with UPLC, a wide variety of flavonoids contained in the HEPrB was observed. Direct antibacterial activity was observed for the standard strain of *Staphylococcus aureus*, however, the extract showed antagonistic activity or no significance in relation to the antibiotics tested in this study. As for toxicity, the HEPrB did not show significant damage in the proposed model. The results emphasize care when associating the consumption of teas with treatments with antibiotics for clinical use.

1. Introduction

The antibiotic therapy brings na effective effect against bacterial infections, controling bacterial diseases previously considered without cure. However, due the uncontroled usage of these drugs, the surveillance of drug resistant bacteria becomes a worldwide problem of public health [1].

These resistance can be due diferente mechanisms as target modified structures, inactivation of the antibiotic action and others, and all of these mechanisms can be transmitted to other bacteria by horizontal gene transference [2].

Bacterial resistance prevents many antibiotics for clinical use from being effective in treating infections, and the search for new alternatives for antibacterial treatments is necessary [3]. By this fact, the search for new antibiotics and ajuvants for these drugs is very importante to face the bacterial resitance to drugs [4].

Based in this situation, the studies seeking secondary metabollites from plants to combat the bacterial resistance to antibiotics as na adjuvante or as na antibacterial agente with direct action are more and more importante [5–10]. Therefore, the use of medicinal plants as a

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source of these new alternatives is emphasized, which may be a promising approach [11].

Plathymenia reticulata Benth, from the Fabaceae family, is popularly known as vinhatica, has high economic value due to the quality of the wood, as well as it is used for the recovery of degraded areas [12]. This species is common in the cerrado and is rich in phenolic compounds, such as tannins and flavonoids [13]. Studies point out extracts of *Plathymenia reticulata* associated with glycemic control and cholesterol [13], protection against mercury toxicity [14] and against local inflammations caused by the Jararaca (Bothrops atrox) poison [15]

This study tested the hydroethanolic extract of the *Plathymenia reticulata* bark (HEPrB) for direct antibacterial activity, in standard strains (*Staphylococcus aureus* 25923, *Pseudomonas aeruginosa* 9027 and *Escherichia coli* 25922) and in multidrug-resistant strains (*Staphylococcus aureus* 10, *Pseudomonas aeruginosa* 03 and *Escherichia coli* 06), its modulatory activity associated with antibiotics for clinical use and toxicity in tests with *Drosophila melanogaster* flies.

2. Material and methods

2.1. Collection and preparation of botanical material

The bark of *Plathymenia reticulata* were collected in Serra do Boqueirão, in a private property, in an amazing state of preservation, in the municipality of Lavras da Mangabeira-CE, according to the coordinates 6° 72′24″S; 38° 97′73″W, at an elevation that varies between 282 and 401 m above sea level. An extract from this plant was deposited at the Herbarium Caririense Dárdano de Andrade Lima −HCDAL, Universidade Regional do Cariri - URCA, under the number 13,692.

The collected material was cut into pieces of ±10 cm and immersed in 70 % ethyl alcohol for 72 h, in the proportion of 10 mL of alcohol to 1 g of crushed skin [16]. After filtration, the liquid obtained was mixed with water in a 1: 1 ratio. This procedure was necessary for lyophilization of the extract in a Mini-spray dryer MSDi 1.0 (Labmaq do Brasil), with a 1.2 mm spray nozzle.

2.2. Plant extract and antibiotics for clinical use

The concentration used in all products in this clinical trial was equivalent to 1,024 μg / mL. To obtain these solutions, 10 mg of each sample was weighed and diluted in 0.5 mL of Dimethylsulfoxide (DMSO) to guarantee complete dilution. Subsequently, the resulting solutions were diluted in distilled water until the desired concentration was obtained.

2.3. UPLC-QTOF-MS / MS analysis

The instrumental analysis of the filtered samples of the *Plathymenia reticulata* extract was performed on an ACQUITY UPLC BEH column (150 × 2.1 mm, 1.7 hm, Waters Co.) in a Waters Acquity UPLC system coupled to a Q-TOF Premier mass spectrometer (Waters MS Technologies, Manchester, United Kingdom). MS data were collected for m / z values in the range of 110−1180 Da. The exact mass and molecular formula assignments were obtained using MassLynx 4.1software (Waters MS Technologies).

2.4. Bacterial strains

In the tests, multi-resistant strains of *Staphylococcus aureus* 10, *Pseudomonas aeruginosa* 03 and *Escherichia coli* 06 were used; and standard strains of *Staphylococcus aureus* 25923, *Pseudomonas aeruginosa* 9027 and *Escherichia coli* 25922, all cultivated at the Microbiology and Molecular Biology Laboratory (LMBM) of the Regional University of Cariri (URCA).

According to the resistance profile, all multiresistant strains used in this test are resistant to ciprofloxacin and levofloxacin. The strain of *Staphylococcus aureus* 10 is also resistant to cephalexin.

2.5. Direct antibacterial activity test

In this test, the methodology proposed by Javadpour et al. [17] was adapted to be used in broth microdilution tests. The bacterial inoculants were grown on HIA (Heart Infusion Agar), growing for 24 h. A strain drag was diluted in 0.9 % Saline (SF), contained in a test tube, with turbidity measured according to the McFarland 0.5 scale (1.5×10^8 UFC/mL). A BHI (Brain Heart Infusion Broth) solution and 10 % of the bacterial inoculum was distributed in eppendorfs microtubes, in a total volume of 1 mL, to be used in the test. This procedure was performed in triplicate for each strain tested.

The BHI solution and inoculum were distributed in 96-well plates, and subsequently microdiluted in HEPrB at a ratio of 1:1. HEPrB concentrations in the wells decline by half the previous well, ranging from 512 μg/mL to 8 μg/mL. After 24 h of incubation in a bacteriology oven at a temperature of 37 °C, the reading of the Minimum Inhibitory Concentration (MIC) was performed using the colorimetric method, using Resazurin (400 μg/mL solution for use (5.33 μg/mL by well).

2.6. Antibiotic action modification test

For this test, eppendorfs microtubes, in a total volume of 1,5 mL, were prepared as described in the previous subchapter. However, the HEPrB was added to the final solution at a sub-inhibitory concentration (CIM/8), according to the methodology described by Coutinho et al. [18]. Serial microdilution was performed using antibiotics for clinical use, and the concentration dropped from 512 μg/mL to 0.5 μg/mL.

In order to try to infer a mechanism of action for the HEPrB, a control was added, in sub-inhibitory concentrations, with the Efflux Pump Standard Inhibitor (EPSI) Clopromazine (CPZ).

2.7. Toxicity test on Drosophila melanogaster

The toxicity test was carried out by exposing flies to the HEPrB in two tests: Mortality test, according to the model proposed by Cunha et al. [19], and the negative geotaxis test, proposed by Coulom & Birman (2004).

In the mortality test, twenty adult flies, four days old, were isolated in pots containing filter paper soaked in a solution of sucrose, extract and distilled water. Concentrations of 50, 25, 10 μg / mL and a 20 % sucrose control were used. This test was repeated in six times for each concentration. Then, mortality readings were taken at time intervals of 3, 6, 9, 12, 24, 36 and 48 h. 12 h light / dark cycle and temperature controlled at 25 °C were respected during the tests.

For the negative geotaxis test, the glass tube in which the flies were contained was tapped so that the flies were positioned at the bottom of the tube. Then the number of flies that were able to climb up to the edges to a height of 4 cm was counted. The reading was performed twice for each tube, following the same time intervals as the mortality test.

2.8. Statistical analysis

In microbiological tests, the triplicate values were used to determine the central values, using geometric means. The central data for the survival test in the *Drosophila melanogaster* model were obtained as an arithmetic mean of the values in each glass tube (n), by concentration and measured hours. For the negative geotaxis tests, an arithmetic average of the two consecutive observations was performed in each glass tube, this being the value of “n”. As the tests were performed in sextuplicate, an average with all "n" was obtained with their respective standard deviations, per hour and measured concentrations. These were the central data.

The central data were submitted to a two-way ANOVA with post hoc Bonferroni test ($p < 0.05$) and analyzed using the statistical program

GraphPad Prisma 5.0.

3. Results and discussion

3.1. UPLC-QTOF-MS/MS analysis

In the identification by UPLC–ESI-QTOF-MS/MS of the HEPrB, sixteen chemical substances were found, as shown in Fig. 1 and Table 1.

Of the sixteen substances found, eight are flavonoids (1,2,5,7,8,14,15,16) and an anthocyanin (3). Study by Wang et al. [23] points out that the extract of *Moringa oleifera*, rich in flavonoid glycosides of quercetin and kaempferol, has high antioxidant activity, while Zhang et al. [24] observed the correlation between antioxidant activity and concentrations of anthocyanins, flavonoids and polyphenols found in three colors of onions (*Allium cepa* L.). Such properties are described in the literature for the flavonoid Kaempferol (14), which besides the mentioned therapeutic property, still demonstrates anti-cancer and anti-inflammatory activities [25].

In the composition of the HEPrB, two fatty acids (9,10) and another phenolic compound from the family of stilbenes (11) were also identified. Adili et al. [26] point out the importance of omega-3 and omega-6 fatty acids in platelet regulation and combating cardiovascular diseases. The octadecadienico acid (linoleic) found in the composition of this extract is implicated in chronic inflammatory processes and obesity [27], however, salts of this acid (Linoleic) are related to the decrease in the skin microbiota, producing a protective effect against several species of the genus *Staphylococcus* [28].

Reinheimer et al. [29], when investigating the action of synthetic isopropylstilbene derivatives on strains of *M. tuberculosis* ATCC 27294 and clinical isolates of multidrug-resistant *M. tuberculosis*, pointed out the potential of synthetic stylbene derivatives as drugs for the treatment of turboculosis. Regarding the stylbene derivative, Resveratrol (11), the literature points out the antioxidant [30], anticancer [31], cardioprotective [32] and its viability for treatment of Alzheimer's disease [33]. In another study, Resveratrol was able to cause damage to the *Salmonella typhimurium* cell membrane, showing an antibacterial effect [34].

Procyanidin dimers produced from the rhizome of *Bistorta officinalis* showed pro-inflammatory effects and reasonable antibacterial activity, with MICs ranging from 31.25 μg/mL to > 500 μg/mL on Gram-positive strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* [35].

3.2. Direct antibacterial activity

The Minimum Inhibitory concentration values (MIC) for the antibacterial activity of HEPrB are shown in Table 2.

The HEPrB showed antibacterial activity for the standard strain of *Staphylococcus aureus*, with an MIC equal to 256 μg/mL. In the other

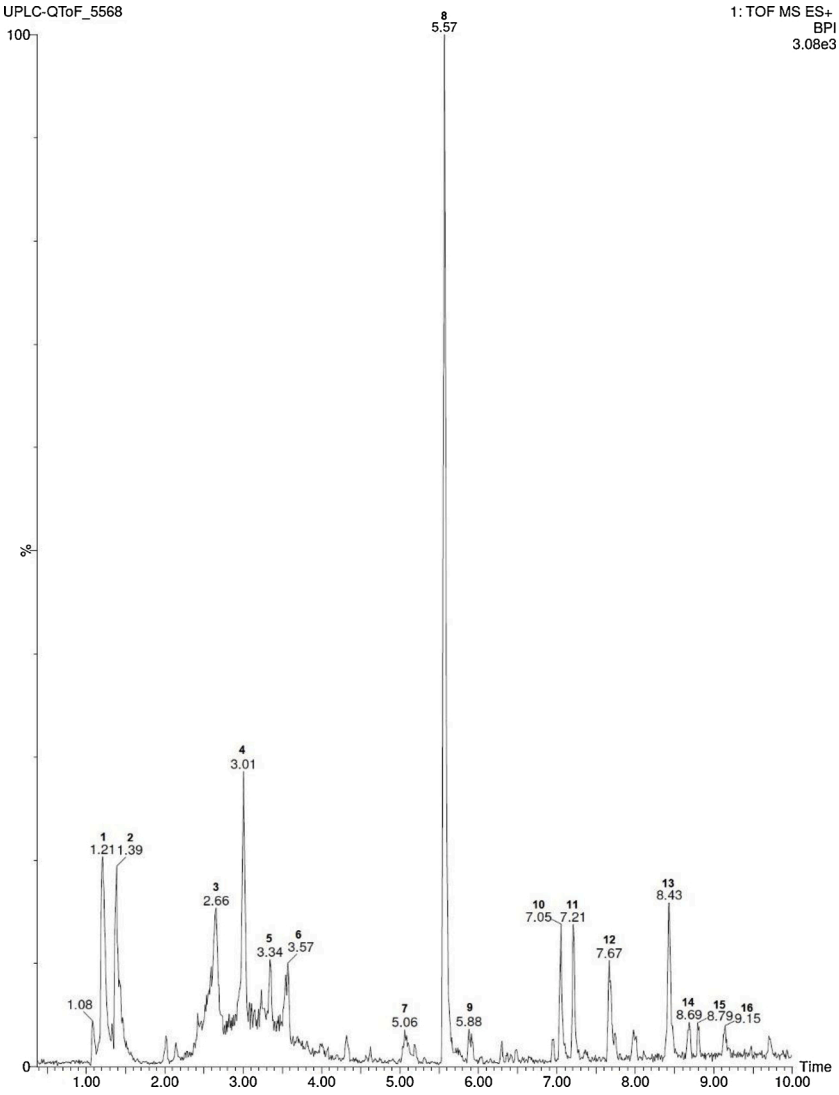

Fig. 1. Chromatogram of high definition mass spectrometry (UPLC-MS) of Hydroethanolic Extract of *Plathymenia reticulata* (HEPrB) in negative ionic mode.

Table 1
UPLC – ESI-QTOF-MSE identification of compounds from the Hydroethanolic Extract of *Plathymenia reticulata* (HEPrB).

Peak no.	Rt min	$[M-H]^-$ Observed	$[M-H]^-$ Calculated	Product Ions (MS/MS)	Empirical Formula	Ppm (error)	Putative Name	References
1	1.21	447.1914	447.1960	285.6300	$C_{21}H_{19}O_{11}$	−10.3	Luteolin-O-hexoside	[20]
2	1.39	611.1411	611.1401	575.1377, 491.1185, 287.0731	$C_{27}H_{31}O_{16}$	1.6	Eriodictyol-di-C-dihexoside	[21]
3	2.66	577.1379	577.1405	425.0864, 407.0660, 289.0590	$C_{30}H_{25}O_{12}$	−4.5	Procyanidin dimer	[20]
4	3.01	595.1482	595.1487	313.0668	$C_{34}H_{43}O_9$	−0.8	Saponin derivative	[20]
5	3.34	579.1511	579.1503	447.0915, 433.0725, 301.0365	$C_{26}H_{27}O_{15}$	1.4	Quercetin pentose deoxyhexose	[21]
6	3.57	139.0402	139.0395	291.0832, 289.0688, 139.0423	$C_7H_7O_3$	5.0	N.i*	–
7	5.06	461.1213	461.1236	299.0892	$C_{22}H_{21}O_{11}$	−5.0	Chrysoeriol-O-hexoside	[20]
8	5.57	755.4483	755.4464	609.1514, 446.0828	$C_{33}H_{39}O_{20}$	2.5	Quercetin di-deoxyhexose hexose	[21]
9	5.88	299.0859	299.0861	–	$C_{18}H_{35}O_3$	−0.7	Hydroxyoctadecanoic acid	[20]
10	7.05	239.0938	239.0927	211.8170	$C_{18}H_{31}O_2$	3.9	Octadecadienoic acid (linoleic)	[20]
11	7.21	227.2033	227.2011	185.0616, 143.0547	$C_{14}H_{11}O_3$	9.7	Resveratrol	[21]
12	7.67	274.2757	274.2746	299.2599, 274.2696, 133.0907	$C_{16}H_{36}NO_2$	4.0	N.i	–
13	8.43	119.0875	119.0861	135.0822, 119.0884	C_9H_{11}	11.8	N.i	–
14	8.69	287.1967	287.2011	151.0073, 133.0281	$C_{15}H_{10}O_6$	−4.0	Kaempferol	[22]
15	8.79	463.2649	463.2637	300.0287, 301.0362	$C_{21}H_{19}O_{12}$	2.6	Quercetin hexose I	[21]
16	9.15	625.3545	625.3529	463.0845, 317.0283, 316.0156	$C_{27}H_{29}O_{17}$	2.6	Myricetin hexose deoxyhexose III	[21]

* N.i - No identified.

Table 2
Minimum Inhibitory Concentration Values (μg/mL).

Substances	Tested Bacteria					
	S.A. 25923	E.C. 25922	P.A. 9027	S.A. 10	E.C. 06	P.A. 03
HEPrB	256	≥1024	≥1024	≥1024	≥1024	≥1024

HEPrB = Hydroethanolic Extract of *Plathymenia reticulata* Bark; S.A., *Staphylococcus aureus;* P.A., *Pseudomonas aeruginosa;* E.C., *Escherichia coli.*

strains, MIC was ≥ 1024 μg/mL, being therefore considered insignificant for in vivo effects, that is, irrelevant for clinical use [36].

Although the result for the *Staphylococcus aureus* strain has a value within a clinically acceptable MIC, for the extract to have a promising clinical importance, this value should be below 100 μg / mL [37].

Studies by Fernandes et al. [38] point out the antibacterial activity of *Plathymenia reticulata* for most Gram-positive bacteria using concentrations of 625 μg/mL, as well as the inability to inhibit some gram-negative bacteria tested by the authors. Ramos et al. [39] observed the activity of *Plathymenia foliolosa*, against *Mycobacterium tuberculosis* and *Mycobacterium kansasii*, with MICs between 0.78 μg/mL and 100 μg/mL, respectively.

The anti-*Staphylococcus* effect of natural products is known in the literature, corresponding with the interaction of the natural products with the cell mambrane, altering the electric potential and the structure of this membrane [40,41].

Decoction, infusion and hydroalcoholic extract rich in flavonoids, some also present in the composition of HEPrB (Quercetin hexose, Kaempferol and Luteolin-O-hexoside), produced from leaves of *Thymus vulgaris* L., were tested for their antioxidant and antibacterial capacity, being verified a high antioxidant power from the DPPH test, as well as a direct antibacterial action on Gram-positive and Gram-negative strains [42].

Partially similar results were obtained by Bitis et al. [43], when testing different extracts of Rosa sempervirens L. in strains of *Staphylococcus aureus*; *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneuminiae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. In the study in question, the extracts had a high content of flavonoids and other phenolic compounds, exhibiting potent antioxidant activity, in addition to not showing antibacterial action against any strain of the study.

Direct antibacterial activity of extracts with a high amount of phenolic compounds was correlated to the content of total phenolic compounds, as well as to the content of their non-flavonoid fraction. Greater effectiveness of these extracts on Gram-positive strains has also been reported [44].

These facts corroborate with the results presented so far, with the divergences possibly resulting from the total phenolic composition, the flavonoid composition and the non-flavonoid content.

3.3. Antibiotic action modifying effect

The modulating effect of the antibiotic activity of HEPrB with Ciprofloxacin and Levofloxacin can be seen in Fig. 2.

Chlorpromazine is a standard efflux pump (EP) inhibitor, widely used in similar experiments to detect this resistance mechanism [45,46]. The results demonstrate an inhibition of EP when the standard inhibitor is associated with the antibiotic, resulting in a MIC less than that of the control, thus proving the presence of EP in the three strains tested for ciprofloxacin, as can be seen in Fig. 2A. When the associated antibiotic is replaced by Levofloxacin (Fig. 2B), we find an absence of EP. The values are informed in the Table 3.

In both situations, the HEPrB presents either antagonistic action or no interaction on the effect of the antibiotic, visualized through a MIC higher than the control or without statistical significance. We can infer that the extract is not able to harm EP and act as a mechanism for altering antibiotic resistance.

HEPrB also impaired the action of antibiotics even though there is no specified mechanism of resistance. Thus, the antagonisms presented lead us to suspect that the extract was not only unable to inhibit the resistance mechanisms, but also impaired some mechanism of action of the antibiotic.

Studies suggest that in addition to the specific mechanism of each antibiotic, its pro-oxidant effect is capable of increasing its effectiveness against microorganisms [47,48] and that the presence of antioxidant substances in the environment interferes in this pro-oxidative effect of antibiotics [48].

Studies with Tetraena simplex, a plant folkly used to the asthma treatment, demonstrated a high antioxidante effect and no antibacterial activity related to the extracts, incluuding among the assayed strains

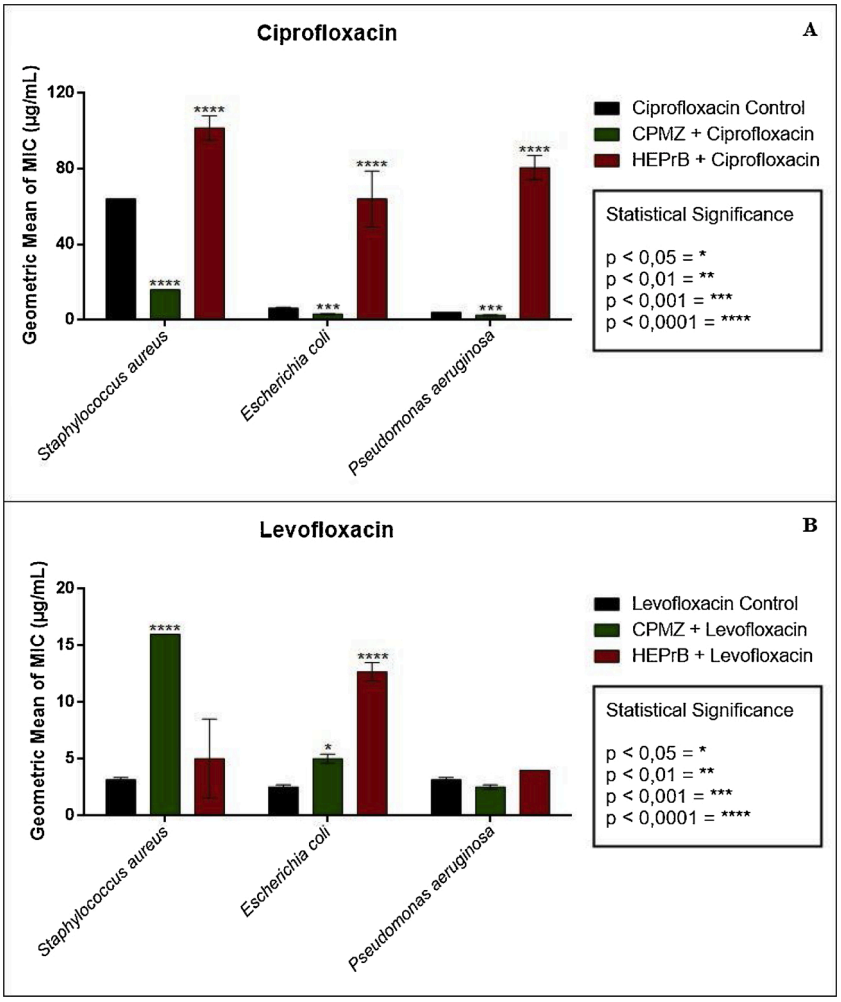

Fig. 2. Effect of the antibiotic modifying action of HEPrB on Gram-positive and Gram-negative strains.
Combined action of the extract with the antibiotic Ciprofloxacin (A); Combined action of the extract with the antibiotic Levofloxacin (B); CPMZ = Chlorpromazine; HEPrB = Hydroethanolic Extract of *Plathymenia reticulata* Bark; * = Represents the statistical significance of a bar when compared to antibiotic control.

Table 3
MIc values of the substances and drugs asayed alone and in association.

	MIC (µg/mL)		
	SA 10	EC 06	PA 24
Ciprofloxacin			
+ CPMZ	16 (4)	3.17 (2)	2.51 (1.6)
+ HEPrB	101.59 (1,6)	64 (10.1)	80.63 (20.2)
Levofloxacin			
+ CPMZ	16 (5)	5.04 (2)	2.52 (1.3)
+ HEPrB	5.04 (1.6)	12.7 (5)	4 (1.3)

CPMZ: Clorpromazine; HEPrB: hydroethanolic extract of the *Plathymenia reticulata* bark; SA: *Staphylococcus aureus*; EC: *Escherichia coli*; PA: *Pseudomonas aeruginosa*; Between the parenthesis is demonstrated the the times reduction or enhanced observed.

Staphyloccocus aureus and *Escherichia coli* [49].

A high rate of phenolic compounds in an extract is indicative of its high antioxidant capacity [50]. In this context, the numerous presence of phenolic compounds and flavonoids in HEPrB may have impaired the action of antibiotics during the modulation test, as it affects their pro-oxidative effect, thus explaining antagonisms.

Another hypothesis for antagonisms would be the large number of interactions between the various compounds of the extract with each other and with the antibiotic, disturbing the structure of the antibiotic, and with it, its action.

Bark extract of *Ziziphus joazeiro* Mart., Which composition had numerous flavonoid compounds, was tested against 3 g-positive strains (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*), presenting 8 antagonistic results out of 9 possible [8]. On the other hand, a study with isolated flavonoids derived from plants demonstrated a high modulatory capacity on the *S. aureus* strain. Some flavonoids were unable to effectively modulate the antibiotic used due to structural differences [51].

The fact that there is a difference between the flavonoids found in the HEPrB and the studies in question may explain the different results found, as well as the interaction of numerous compounds in the extract may end to interfere in a promising action of a compound that alone would have an interesting action, explaining the lack of bioactivity of the extract on the strains.

3.4. HEPrB toxicity test

The toxicity test on *Drosophila melanogaster* flies is a viable model for the verification of toxic activities of substances [52,53]. Toxicity is verified through mortality and the effect of the extract on the flies' locomotor system. Mortality is verified according to the number of fly deaths when they are in contact with different concentrations of the extract, while the deficiency under the locomotor system is indicative of a possible disabling injury.

The results obtained on the mortality and damage to the locomotor apparatus of *D. melanogaster* flies caused by the action of the HEPrB are contained in Fig. 3.

In the mortality test, the extract had no potential to kill flies. It is unlikely that the death that occurred after 24 h was due to the HEPrB. As for the negative geotaxis test, damage to the locomotor system was observed after 36 h of exposure to the extract in the highest concentration produced (50 μg/mL), corroborating the low mortality. From the results presented, we can infer that the tested extract does not present high toxicity.

Tests on the toxicity and analgesic activity of the flavonoids of *Achillea wilhelmsii* and *Teucrium stocksianum* in mice, showed the safety of the action of the flavonoids in doses of up to 100 mg / kg, with prominent lethality in doses of 1,000 mg/kg [54].

Cunha et al. [55], when investigating the cytoprotective effect of *Eugenia uniflora* L. extract against mercury chloride contamination, observed that the extract that had phenols and flavonoids in its composition (1.38 % quercetin, 1.17 % of quecitrin and 1.16 % of ellagic acid) presented low toxicity when tested in *Drosophila melanogaster*.

Extracts of *Cassia fistula* Linn. Demonstrated the presence of phenolic compounds and presented an antibacterial effect against strains of S. typhosa and *S. dysenteriae,* with a low in vivo toxicity [56], as demonstrated by our study. Weli et al. [57], working with extracts from banana, demonstrated a correlation between a higher polaity of the solvente and higher toxocity against *Artemia salina* and antibacterial acivity. This effect about the polarity of the solvent reinforce our results and putative effect of flavonoids in the observed activities againts the antibiotic activity and in the low toxicity using the *Drosophila melanogaster* model.

4. Conclusion

HEPrB presented in its composition numerous phenolic compounds, mostly flavonoids. Some of these flavonoids, in isolation, have numerous biological activities, highlighting the antioxidant activity, characteristic of this group of compounds. Antibacterial activity is also reported in the scientific literature.

HEPrB showed direct antibacterial activity only for the standard strain of *Staphylococcus aureus*, however it did not show any activity to modify bacterial resistance. A probable explanation would be the interference of the extract in some mechanism of action of the antibiotic, notably in the pro-oxidative mechanism, caused by the antioxidant nature of this class of compounds.

The extract also showed low toxicity according to the proposed model.

The result of this research shows the care in relation to the use of teas during treatment with antibiotics, a common activity in popular medicine, since plant extracts when associated with antibiotics for clinical use, can harm the action of the medication.

Author statement

E.M. Luna – Methodology.
T.S. Freitas – Validation.
F.F. Campina – Methodology.
M.S. Costa – Methodology.
J.E. Rocha – Validation.
R.P. Cruz – Validation.
D.L.S. Júnior – Software.
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J.C.P. Andrade – Resources.
F.N. Pereira-Júnior – Resources.
M.A.N. Lisboa – First draft of the manuscript.
G.V. Cruz – First draft of the manuscript.
J.T. Calixto Júnior - supervision; Final draft of the manuscript.
A.M.R. Teixeira – supervision; Final draft of the manuscript.
H.D.M. Coutinho – coordination of the project.

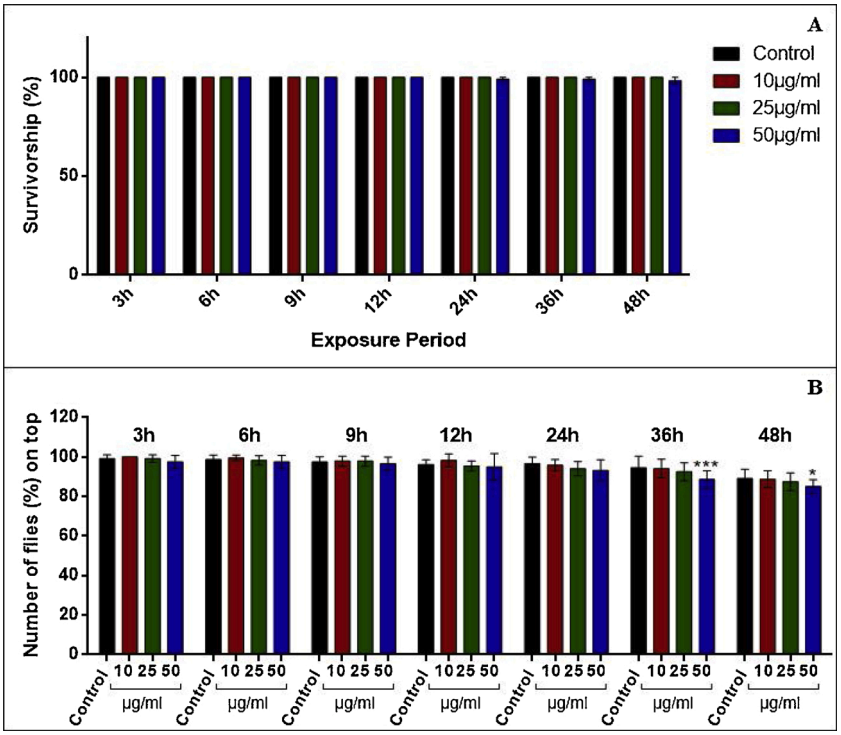

Fig. 3. HEPrB toxicity on *Drosophila melanogaster* flies.
Mortality caused by HEPrB on *Drosophila melanogaster* (A) flies; Damage caused by the HEPrB to the locomotor apparatus of the flies (B); * = Represents the statistical significance of a bar when compared to antibiotic control.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

[1] K.H. Luepke, K.J. Suda, H. Boucher, R.L. Russo, M.W. Bonney, T.D. Hunt, J. F. Mohr I.I.I., Past, present, and future of antibacterial economics: increasing bacterial resistance, limited antibiotic pipeline, and societal implications, Pharm. J. Human Pharmacol. Drug Therapy 37 (1) (2017) 71–84.

[2] I. Sultan, S. Rahman, A.T. Jan, M.T. Siddiqui, A.H. Mondal, Q.M.R. Haq, Antibiotics, resistome and resistance mechanisms: a bacterial perspective, Front. Microbiol. 9 (2018) 2066.

[3] M. Masoumian, M. Zandi, Antimicrobial activity of some medicinal plant extracts against multidrug resistant bacteria, Zahedan J. Res. Med. Sci. 19 (11) (2017).

[4] C. González-Bello, Antibiotic adjuvants–A strategy to unlock bacterial resistance to antibiotics, Bioorg. Med. Chem. Lett. 27 (18) (2017) 4221–4228.

[5] R.P. Mittal, V. Jaitak, Plant-derived natural alkaloids as new antimicrobial and adjuvant agents in existing antimicrobial therapy, Curr. Drug Targets 20 (14) (2019) 1409–1433.

[6] M.D.S. Costa, N.J. Santos Araújo, T.S. de Freitas, F.A.B.D. Cunha, W.D. Amaral, C. Deschamps, et al., GC-FID analysis and antibacterial activity of the calyptranthes concinna essential oil against MDR bacterial strains, Separations 7 (1) (2020) 10.

[7] T.A. Andrade, T.S. Freitas, F.O. Araújo, P.P. Menezes, G.A.A. Dória, A.S. Rabelo, et al., Physico-chemical characterization and antibacterial activity of inclusion complexes of Hyptis martiusii Benth essential oil in β-cyclodextrin, Biomed. Pharmacother. 89 (2017) 201–207.

[8] J.C. Andrade, A.R.P. Silva, A.T.L. Santos, M.A. Freitas, J.N.P. Carneiro, M.I. P. Gonçalo, et al., UPLC-MS-ESI-QTOF characterization and evaluation of the antibacterial and modulatory antibiotic activity of Ziziphus joazeiro Mart. Aqueous extracts, South Afr. J. Bot. 123 (2019) 105–112.

[9] R.O.M. Silva, J.W.G. Castro, O.D.M.D. Junior, A.C.J. de Araújo, M.K.D.N. S. Leandro, R.J.O. Costa, et al., Photoinduced Antibacterial Activity of the Essential Oils from Eugenia brasiliensis Lam and Piper mosenii C. DC. by Blue Led Light, Antibiotics 8 (4) (2019).

[10] Z.S. Silveira, N.S. Macêdo, T.S. de Freitas, A.R.P. da Silva, J.F.S. dos Santos, M.F. B. Morais-Braga, et al., Antibacterial enhancement of antibiotic activity by Enterolobium contortisiliquum (Vell.) Morong, Asian Pac. J. Trop. Biomed. 7 (10) (2017) 945–949.

[11] S.D. Jastaniah, The antimicrobial activity of some plant extracts, commonly used by Saudi people, against multidrug resistant bacteria, Life Sci. J. 11 (8) (2014) 78–84.

[12] L.C. Souza, A.L. da Silva Júnior, F.D. de Miranda, M.C. de Souza, S. Horn Kunz, A. G. Pereira, Validação do marcador molecular ISSR para detecção de diversidade genética em *Plathymenia reticulata* Benth, Brazilian Journal of Agricultural Sciences/Revista Brasileira de Ciências Agrárias 13 (1) (2018).

[13] F.O. Magalhães, E. Uber-Bucek, P.I.B. Ceron, H.E. Coelho, C.H.G. Barbosa, T. Carvalho, M. Groppo, Tratamento crônico com extrato hidroalcoólico de *Plathymenia reticulata* promove hiperplasia de ilhotas e controle glicêmico em ratos diabéticos, Einstein (São Paulo) 17 (3) (2019).

[14] K. Gombeau, R.B. De Oliveira, S.L.F. Sarrazin, R.H.V. Mourão, J.P. Bourdineaud, Protective effects of *Plathymenia reticulata* and Connarus favosus aqueous extracts against cadmium-and mercury-induced toxicities, Toxicol. Res. 35 (1) (2019) 25–35.

[15] V.M. de Moura, W.C.R. da Silva, J.D. Raposo, L.A. Freitas-de-Sousa, M.C. Dos-Santos, R.B. de Oliveira, R.H.V. Mourão, The inhibitory potential of the condensed-tannin-rich fraction of *Plathymenia reticulata* Benth.(Fabaceae) against Bothrops atrox envenomation, J. Ethnopharmacol. 183 (2016) 136–142.

[16] B.G. Brasileiro, V.R. Pizziolo, D.S. Raslan, C.M. Jamal, D. Silveira, Antimicrobial and cytotoxic activities screening of some Brazilian medicinal plants used in Governador Valadares district, Revista Brasileira de Ciências Farmacêuticas 42 (2) (2006) 195–202.

[17] M.M. Javadpour, M.M. Juban, W.C.J. Lo, S.M. Bishop, J.B. Alberty, S.M. Cowell, et al., De novo antimicrobial peptides with low mammalian cell toxicity, J. Med. Chem. 39 (16) (1996) 3107–3113.

[18] H.D. Coutinho, J.G. Costa, J.P. Siqueira-Júnior, E.O. Lima, In vitro anti-staphylococcal activity of Hyptis martiusii Benth against methicillin-resistant *Staphylococcus aureus*: MRSA strains, Revista Brasileira de Farmacognosia 18 (2008) 670–675.

[19] F.A.B. Cunha, G.L. Wallau, A.I. Pinho, M.E.M. Nunes, N.F. Leite, S.R. Tintino, et al., Eugenia uniflora leaves essential oil induces toxicity in *Drosophila melanogaster*: involvement of oxidative stress mechanisms, Toxicol. Res. 4 (3) (2015) 634–644.

[20] A.Faraga Mohamed, Amira R. Khattabc, Amal A. Maamound, Matthias Kropfe, Andreas G. Heissf, UPLC-MS metabolome based classification of Lupinus and Lens seeds: a prospect for phyto-equivalency of its different accessions, Food Res. Int. 115 (2019) 379–392.

[21] M.Abu-Reidah Ibrahim, María del Mar Contreras, David Arráez-Román, Alberto Fernández-Gutiérrez, Antonio Segura-Carretero, UHPLC-ESI-QTOF-MS-based metabolic profiling of Vicia faba L. (Fabaceae) seeds as a key strategy for characterization in foodomics, Electrophoresis 35 (2014) 1571–1581.

[22] Avulaa Bharathi, Yan-Hong Wanga, Troy J. Smilliea, Xiang Fua, Xing Cong Li, Wilfred Mabuselac, James Sycec, Quinton Johnsonc, William Folkd, Ikhlas A. Khan, Quantitative determination of flavonoids and cycloartanol glycosides from aerial parts of Sutherlandia frutescens (L.) R. BR. by using LC-UV/ELSD methods and confirmation by using LC–MS method, J. Pharm. Biomed. Anal. 52 (2010) 173–180.

[23] Y. Wang, Y. Gao, H. Ding, S. Liu, X. Han, J. Gui, D. Liu, Subcritical ethanol extraction of flavonoids from Moringa oleifera leaf and evaluation of antioxidant activity, Food Chem. 218 (2017) 152–158.

[24] S.L. Zhang, D.E.N.G. Peng, Y.C. Xu, S.W. Lü, J.J. Wang, Quantification and analysis of anthocyanin and flavonoids compositions, and antioxidant activities in onions with three different colors, J. Integr. Agric. 15 (9) (2016) 2175–2181.

[25] M. Imran, A. Rauf, Z.A. Shah, F. Saeed, A. Imran, M.U. Arshad, et al., Chemo-preventive and therapeutic effect of the dietary flavonoid kaempferol: a comprehensive review, Phytother. Res. 33 (2) (2019) 263–275.

[26] R. Adili, M. Hawley, M. Holinstat, Regulation of Platelet Function and Thrombosis by omega-3 and omega-6 Polyunsaturated Fatty Acids, Prostaglandins & Other Lipid Mediators, 2018.

[27] S.S. Naughton, M.L. Mathai, D.H. Hryciw, A.J. McAinch, Linoleic acid and the pathogenesis of obesity, Prostaglandins Other Lipid Mediat. 125 (2016) 90–99.

[28] H. Jang, Y. Makita, K. Jung, S. Ishizaka, K. Karasawa, K. Oida, et al., Linoleic acid salt with ultrapure soft water as an antibacterial combination against dermato-pathogenic Staphylococcus spp, J. Appl. Microbiol. 120 (2) (2016) 280–288.

[29] C. Reinheimer, D. Büttner, E. Proschak, H.B. Bode, V.A. Kempf, T.A. Wichelhaus, Anti-tubercular activity of a natural stilbene and its synthetic derivatives, GMS Infect. Dis. (2018) 6.

[30] N. Xia, A. Daiber, U. Förstermann, H. Li, *Antioxidant effects of resveratrol in the cardiovascular system*, Br. J. Pharmacol. 174 (12) (2016) 1633–1646.

[31] E.M. Varoni, A.F. Lo Faro, J. Sharifi-Rad, M. Iriti, Anticancer molecular mechanisms of resveratrol, Front. Nutr. 3 (2016) 8.

[32] A. Riba, L. Deres, B. Sumegi, K. Toth, E. Szabados, R. Halmosi, Cardioprotective effect of resveratrol in a postinfarction heart failure model, Oxid. Med. Cell. Longev. 2017 (2017) 1–10.

[33] C. Moussa, M. Hebron, X. Huang, J. Ahn, R.A. Rissman, P.S. Aisen, R.S. Turner, Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's disease, J. Neuroinflammation 14 (1) (2017).

[34] W. Lee, D.G. Lee, Resveratrol induces membrane and DNA disruption via pro-oxidant activity against Salmonella typhimurium, Biochem. Biophys. Res. Commun. 489 (2) (2017) 228–234.

[35] K.A. Pawłowska, R. Hałasa, M.K. Dudek, M. Majdan, K. Jankowska, S. Granica, Antibacterial and anti-inflammatory activity of bistort (Bistorta officinalis) aqueous extract and its major components. Justification of the usage of the medicinal plant material as a traditional topical agent, J. Ethnopharmacol. (2020), 113077.

[36] P.J. Houghton, M.J. Howes, C.C. Lee, G. Steventon, Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant, J. Ethnopharmacol. 110 (3) (2007) 391–400.

[37] J.L. Rios, M.C. Recio, Medicinal plants and antimicrobial activity, J. Ethnopharmacol. 100 (1–2) (2005) 80–84.

[38] T.T. Fernandes, A.T. dos Santos, F.C. Pimenta, Atividade antimicrobiana das plantas *Plathymenia reticulata*, Hymenaea courbaril e Guazuma ulmifolia, Revista de Patologia Tropical/Journal of Tropical Pathology 34 (2) (2005).

[39] D.F. Ramos, G.G. Leitão, F.D.N. Costa, L. Abreu, J.V. Villarreal, S.G. Leitão, et al., Investigation of the antimycobacterial activity of 36 plant extracts from the brazilian Atlantic Forest, Revista Brasileira de Ciências Farmacêuticas 44 (4) (2008) 669–674.

[40] W. Hu, C. Li, J. Dai, H. Cui, L. Lin, Antibacterial activity and mechanism of Litsea cubeba essential oil against methicillin-resistant Staphylococcus aureus (MRSA), Ind. Crops Prod. 130 (2019) 34–41.

[41] F.D. Gonelimali, J. Lin, W. Miao, J. Xuan, F. Charles, M. Chen, S.R. Hatab, Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms, Front. Microbiol. 9 (2018) 1639.

[42] N. Martins, L. Barros, C. Santos-Buelga, S. Silva, M. Henriques, I.C. Ferreira, Decoction, infusion and hydroalcoholic extract of cultivated thyme: antioxidant and antibacterial activities, and phenolic characterisation, Food Chem. 167 (2015) 131–137.

[43] L. Bitis, A. Sen, N. Ozsoy, S. Birteksoz-Tan, S. Kultur, G. Melikoglu, Flavonoids and biological activities of various extracts from Rosa sempervirens leaves, Biotechnol. Biotechnol. Equip. 31 (2) (2017) 299–303.

[44] Y. Tian, A. Puganen, H.L. Alakomi, A. Uusitupa, M. Saarela, B. Yang, Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants, Food Res. Int. 106 (2018) 291–303.

[45] S. Prasch, A.G. Duran, N. Chinchilla, J.M. Molinillo, F.A. Macías, F. Bucar, Resistance modulatory and efflux-inhibitory activities of capsaicinoids and capsinoids, Bioorg. Chem. 82 (2019) 378–384.

[46] G. Li, J. Zhang, C. Li, Q. Guo, Y. Jiang, J. Wei, et al., Antimycobacterial activity of five efflux pump inhibitors against Mycobacterium tuberculosis clinical isolates, J. Antibiot. 69 (3) (2016) 173–175.

[47] P. Belenky, J.J. Collins, Antioxidant strategies to tolerate antibiotics, Science 334 (6058) (2011) 915–916.

[48] A.P. Pesarico, G. Sartori, C.F. dos Santos, J.S. Neto, V. Bortolotto, R.C.V. Santos, et al., 2, 2′-Dithienyl diselenide pro-oxidant activity accounts for antibacterial and antifungal activities, Microbiol. Res. 168 (9) (2013) 563–568.

[49] A.M. Abdulsattar, M.A. Hossain, Antibacterial and antioxidant potential of Tetraena simplex extracts of various polarities, Toxicol. Rep. 7 (2020) 925–929.

[50] M.I. Dias, L. Barros, R.C. Alves, M.B.P. Oliveira, C. Santos-Buelga, I.C. Ferreira, Nutritional composition, antioxidant activity and phenolic compounds of wild Taraxacum sect. Ruderalia, Food Res. Int. 56 (2014) 266–271.

[51] H.T. Diniz-Silva, M. Magnani, S. de Siqueira, E.L. de Souza, J.P. de Siqueira-Júnior, Fruit flavonoids as modulators of norfloxacin resistance in *Staphylococcus aureus* that overexpresses norA, LWT-Food Sci. Technol. 85 (2017) 324–326.
[52] C.E. Sobral-Souza, N.F. Leite, F.A. Cunha, A.I. Pinho, R.S. Albuquerque, J. N. Carneiro, et al., Cytoprotective effect against mercury chloride and bioinsecticidal activity of Eugenia jambolana Lam, Arab. J. Chem. 7 (1) (2014) 165–170.
[53] F.A.B. Cunha, Toxicidade de Eugenia uniflora L.(myrtaceae) em modelos de Drosophila melanogaster e células sanguíneas humanas Doctoral dissertation, Universidade Federal de Santa Maria, 2015.
[54] N. Ali, U. Sultana, S.W.A. Shah, M. Nabi, S. Ullah, I. Shah, G. Ahmad, Acute toxicity and analgesic activity of crude flavonoids of achillea wilhelmsii and teucrium stocksianum, Khyber Med. Univ. J. 8 (1) (2016).
[55] F.A. Cunha, A.I. Pinho, J.F. Santos, C.E. Sobral-Souza, R.S. Albuquerque, E. F. Matias, et al., Cytoprotective effect of Eugenia uniflora L. Against the waste contaminant mercury chloride, Arab. J. Chem. 12 (8) (2019) 4197–4203.
[56] A.Y. Chaerunisaa, Y. Susilawati, M. Muhaimin, T. Milanda, R. Hendriani, A. Subarnas, Antibacterial activity and subchronic toxicity of Cassia fistula L. Barks in rats, Toxicol. Rep. 7 (2020) 649–657.
[57] A.M. Weli, J.R. AL-Hinai, J.M. Al-Mjrafi, J.R. Alnaaimi, M.A. Hossain, S. Saeed, M. S. Aktar, Effect of different polarities leaves crude extracts of Omani juniperus excels on antioxidant, antimicrobial and cytotoxic activities and their biochemical screening, Asian Pacific J. Reprod. 3 (3) (2014) 218–223.